further define the recombinant complementing gene of (b). A functional replacement for the essential gene of (a), based on the discussion in the specification cited above, is a gene (i.e., the recombinant complementing gene of (b)) that provides *the essential function required for cell viability* that is encoded by the essential gene of (a). Thus, it is clear based on a reading of the specification which function of the essential enzyme [gene (as amended)] will be replaced by the recombinant complementing gene on the vector.

Similarly, with respect to claim 12 as amended, the term 'functional replacement' is clear. When the vector of claim 12, comprising a recombinant complementing gene, is present in a microorganism having a non-functional native chromosomal essential gene, the recombinant complementing gene is a functional replacement for the non-functional native chromosomal essential gene when it provides *the essential function required for cell viability* that is encoded by the essential gene. Again, based on a reading of the specification, it is clear which functions of the essential enzyme [gene (as amended)] will be replaced by the vector.

Claims 3 and 6 are allegedly indefinite for the use of abbreviations for a variety of genes without their full names. This rejection is based on an erroneous interpretation of the language of the claims. The 'abbreviations' referred to in the Office Action are in fact not abbreviations, but rather, *are the names of the genes.* A skilled artisan would readily recognize that the italicized terms in each of claims 3 and 6 are not abbreviations, and are in fact gene names. For further guidance on this point, refer to any of the references cited in the specification at pages 18-19. (For example: Umbarger, chapter 27 in Neidhardt et al. (1996); S.J. O'Brien, ed., GENETIC MAPS 1987, Cold Spring Harbor Laboratories; U.S. Patent Nos: 4,190,495, 5,672,345 and 5,840,483). Therefore, claims 3 and 6 are not indefinite.

Claims 11 and 14 are allegedly unclear for the recitation of abbreviations without the full name or explanation of the abbreviation. Applicants point out that the amendments to claims 11 and 14 incorporate the full names to which the abbreviations refer. Therefore, claims 11 and 14 are not indefinite.

In light of the above discussion and the amendments to the claims, applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

Rejections under 35 U.S.C. §103

Claims 1-22 are rejected under 35 U.S.C. §103(a) as being unpatentable over Nayak et al., (Infection and Immunity 66(8):3744-3751, 1998) in view of Curtiss (U.S. Patent Nos: 5,840,483 and 6,024,961). Applicants point out that all of the above references utilize methods of mutating the native chromosomal essential gene whereby the sequences flanking the gene are deleted. (See materials and methods section of the Nayak paper, and the Examples in each of the Curtiss patents). Therefore, it is not

possible for the recombinant complementing gene of the vector to recombine to replace the non-functional native chromosomal essential gene. This argument was used by the Patent Office as the basis for requiring the limitation in the claims of the Curtiss '483 patent 'wherein the first recombinant gene cannot recombine to replace the defective chromosomal gene.'

Here, in the instant specification, Applicants have provided data (See Examples generally, particularly Examples 1 and 5) that show, surprisingly, that even though the complementing gene can recombine, based on the homology between the vector and the chromosome in the flanking regions, to replace the non-functional native chromosomal essential gene, such a balanced-lethal host-vector system is functional and has utility. The claims of the instant application recite the limitation 'wherein the complementing gene can recombine to replace the non-functional native chromosomal essential gene.' The instant invention would not have been obvious, because if as postulated by the USPTO the recombinant complementing gene recombines to replace the non-functional chromosomal gene, there would not be any selective pressure to maintain the vector, comprising the desired gene, in progeny populations of the microorganism. Here, for the first time, applicants have demonstrated that despite the possibility that the complementing gene present on the recombinant vector can recombine to replace the non-functional native chromosomal essential gene, sufficient selective pressure remains to maintain the vector in progeny populations because such recombination either does not occur, or occurs at frequencies that do not compromise the utility of such balanced-lethal host-vector systems. Without the data presented in the instant application, generated by extensive experimentation, the skilled artisan would not have a reasonable expectation of success in utilizing balanced-lethal host-vector systems in which the complementing gene can recombine to replace the non-functional native chromosomal essential gene. It is impermissible to use hindsight based on the benefit of this new data in rejecting the instant claims as obvious. *In re Dembiczak,* 175 F.3d 994 (Fed. Cir. 1999).

In addition, the specification of the '483 patent teaches that it is preferable that the non-functional native chromosomal essential gene lack homology with the complementing gene on the vector, such that the complementing gene *cannot* recombine to replace the non-functional chromosomal gene. (See specification column 16, lines 23-61). Thus, the '483 patent actually teaches *away* from the instant invention. For this reason, the instant invention is not obvious in view of the cited combination of references. *In re Gurley,* 27 F.3d 551 (Fed. Cir. 1994).

In view of the above remarks, applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §103(a).

It is believed that based on the amendments and arguments presented herein, the claims are in condition for allowance, and therefore applicants request such action. If, however, any issues remain, the Examiner is invited to contact the undersigned.

Respectfully submitted,

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AMENDED CLAIMS

1. (Twice amended) An attenuated derivative of a pathogenic microorganism which comprises:

(a) a non-functional native chromosomal essential gene;

(b) a recombinant complementing gene on an extrachromosomal vector, wherein the complementing gene can recombine to replace the non-functional native chromosomal essential gene; and

(c) a desired gene on the extrachromosomal vector, wherein the desired gene is a recombinant gene encoding a desired gene product;

wherein said complementing gene of (b) is a functional replacement for said essential gene of (a), wherein the desired gene is stably maintained in a progeny population of the microorganism.

B1

Sub C1

3. (Amended) The microorganism of claim 2, wherein the non-functional native chromosomal essential gene is a gene selected from the group consisting of a *pab* gene, a *pur* gene, an *aro* gene, *nadA*, *pncB*, *galE*, *pmi*, *fur*, *rpsL*, *ompR*, *htrA*, *hemA*, *cdt*, *cya*, *crp*, *dam*, *phoP*, *phoQ*, *rfc*, *poxA*, *galU*, *mviA*, *sodC*, *recA*, *ssrA*, *sirA*, *inv*, *hilA*, *rpoE*, *flgM*, *tonB*, and *slyA*.

B2

B3

7. (Amended) The microorganism of claim 6, wherein the non-functional native chromosomal essential gene is an *asd* gene wherein said *asd* gene comprises an insertion or a deletion.

11. (Amended) The microorganism of claim 10, wherein the eukaryotic promoter is a CMV (cytomegalovirus) promoter.

B4

12. (Amended) A recombinant vector comprising a recombinant complementing gene, wherein the recombinant complementing gene lacks an RNA polymerase -35 recognition sequence and a promoter -10 sequence,

wherein the recombinant complementing gene is a functional replacement for a non-functional native chromosomal essential gene when the vector is present in a microorganism having a non-functional native chromosomal essential gene.

13. (Amended) The recombinant vector of claim 12, wherein the vector is a plasmid capable of expressing the recombinant complementing gene in a microorganism that is a member of the *Enterobacteriaceae*.

14. (Amended) The recombinant vector of claim 12, wherein the recombinant complementing gene encodes an enzyme that catalyzes a step in the biosynthesis of DAP (mesodiaminopimelic acid).

15. (Amended) The recombinant vector of claim 14, wherein the recombinant complementing gene is an *asd* gene.

16. (Amended) The recombinant vector of claim 12, further comprising a gene encoding a desired gene product.